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Differential Properties Of Stromal Cells From Bone Marrow, Adipose, Cardiac and Liver

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Bone marrow (BM) stromal cells (also termed mesenchymal stem cells; MSC) have been extensively studied and shown to control differentiation of hematopoietic stem cells (HSCs) in part through secreted growth factors. In addition, MSCs support the growth of tumor cells both in vitro and in vivo. As MSCs are being evaluated in a number of clinical trials for regenerative medicine approaches, it is critical to understand the role of stromal cells in different tissues. Stromal cells were isolated from various human tissues including adipose (Ad), cardiac (Car) and liver (Liv) tissues as well as bone marrow (BM) and had an identical appearance in culture. We have compared the stromal cells from these tissues and demonstrate that although they have similar morphology and surface marker expression (CD105+/CD90+/CD73+ and CD45-/CD34-) they have differential biological properties. BM MSCs have been shown previously to support both normal hematopoietic cells and tumor cells.

To test the functional properties of the stromal cell lines we co-cultured stromal cells with either cord blood (CB) cells or tumor cells (K562). As we have previously reported, co-culture of CB on BM-MSCs resulted in expansion of hematopoietic cells. Similarly, Ad stromal cells supported expansion of hematopoietic cells, however, Liv and Car stromal cells failed to expand the CB cells. The effect of the Car stroma was through a cytostatic mechanism as removal of the CB cells from the stromal cells after 7 days of co-culture resulted in hematopoietic cell expansion and detection of colony forming cells by methycellulose assay. We also tested the potential of the stromal cells to support tumor cell growth by co-culturing K562 cells with the stromal cells. Ad and Liv stroma supported growth of the K562 cells similar to BM MSCs, while Car stromal cells inhibited K562 cell growth with few viable cells after 7 days of culture.

These data suggest that the function of stromal cells from different tissue is variable. In particular, tumor development in cardiac tissue is a rare event and our data suggest that Car stromal cells may play a key role in inhibiting proliferation of tumor cells. Further we propose that non homologous use of stromal cells in clinical applications may have deleterious effects, for example the use of BM-MSCs to repair ischemic tissue in the heart may lead to a tumor supportive environment in cardiac tissue.

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Development of Conditioning Regimens Based on HSC-Targeted Antibody ACK2

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Background: Marrow conditioning prior to HSC transplantation incorporates chemotherapy and/or radiotherapy to eradicate malignant disease, eliminate immunologic barriers to allogeneic cell engraftment, and to 'make space' for donor HSC within the HSC niche. The sequelae of such treatments can be substantial, including direct organ toxicity. Selective targeting of HSC with monoclonal antibodies presents an alternative approach to induce stem cell depletion with fewer off target effects. Previous studies in a congenic mouse transplant model established that antibody-mediated blockade of c-Kit, the receptor for stem cell factor, given in combination with low dose irradiation (LD-IR) efficiently depletes HSC and substantially enhances engraftment of donor HSC in immunocompetent mice (Xue et al, Blood 2010). However, since even low doses of irradiation have long-term risks, particularly in children, an important goal is to develop improved regimens utilizing c-Kit blockade.

Objective: To determine if replacing low dose irradiation (LD-IR) with chemotherapy will synergize with c-Kit blockade and improve engraftment in a congenic mouse transplant model.

Methods: Mice were transplanted according to standard protocol. C57B6 (CD45.2) mice were used as donors, and F1 progeny of C57B6 and BoyJ mice (CD45.1) were used as recipients. Recipient mice were treated with 2 mg of the KIT-blocking monoclonal antibody ACK2 (injected intraperitoneally) combined with various schedules of Fludarabine 100mg/kg IP / Cyclophosphamide 200mg/kg IP (FluCy); or 5-flurouracil (5-FU)150 mg/kg IP; or Busulfan (Bu) 20mg/kg IP. Control mice were treated with the ACK2 and 300 cGy. This was followed by transplantation of 1- 2 million freshly isolated congenic marrow cells and subsequent long-term engraftment analysis by flow cytometry.

Results: In comparison to ACK2/LD-IR, the level of engraftment post-transplant was much lower in all of the other treatment arms. ACK2/FluCy had $< 5\%$ engraftment at 13 weeks compared to 80% engraftment in the ACK2/LD-IR arm. ACK2/Bu had $< 5\%$ engraftment compared to 70% in the ACK2/LD-IR arm at 20 weeks and ACK2/5-FU had $< 5\%$ compared to 80% engraftment at 13 weeks in the ACK2/LDR arm.

Conclusion: Our study demonstrates that combination of antibody-mediated c-Kit blockade and chemotherapy did not enhance congenic donor cell engraftment, at least in the regimens tested. Further experiments to elucidate how LD-IR and c-Kit blockade synergize to deplete HSCs and enhance engraftment are ongoing.

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Experience in a Public Cord Blood Bank Using a Segment-Based Aldehyde Dehydrogenase Assay As a Biomarker for Umbilical Cord Blood Potency

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